Gel Extraction of RFP in PSB1C3

Made with Benchling

Project: Awesome Possum

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Purpose:

Obtain digested purified PSB1C3 Plasmid without RFP fragment using gel electrophoresis.

Materials:

- Digested RFP in PSB1C3 Samples A & B
- Invitrogen 8 well Select Size 2% gel cassette
- E-Gel Base
- Micropipette
- Sterile micropipette tips
- Eppendorf Tube
- dH_20
- GeneRuler 1 Kbp Ladder

Protocol:

The cassette was loaded with 25.0 µL of Sample A (22.0 µL dH_20 and 3 µL DNA) in lanes 1 and 3 and 25.0 µL of Sample B (22.0 µL dH_20 and 3 µL DNA) in lanes 6 and 8. Then 10.0 µL of GeneRuler was added to lane M. After that all empty wells were filled with 25.0 µL of dH_20. The cassette was then loaded into the E-gel base and set to 25.0 mins. Once the largest fragment (purified cut PSB vector) reached the reference point additional dH_20 was added to bottom wells. Next the cassette was allowed to run until the bright green fragments reached the bottom wells. Then, the bright green fragments from wells 1, 3, 6, and 8 were retrieved with a micropipette and combined in a 1.5 mL eppendorf tube.

DNA Concentration of Combined Purified Digested PSB1C3 Vector

After combining the purified samples they were measured for DNA concentration using an Implen P 300 nano-photometer. The nano-photometer was blanked using 1.0 µL of dH_20. Then the concentration was measured using 1.0 µL of the combined sample. The concentration values obtained were noted in Table 1.

Table 1: DNA Concentration of Combined Samples A and B

	A	B	C	D	E	F	G
1	**Sample**	**Concentration (ng/µL)**	**A230**	**A260**	**A280**	**A320**	**A260/A**
2	Combined A and B	140	0.228	0.056	0.044	0.001	1.273